The immunoglobulin heavy chain gene (*IgH*) is composed of a number of variable (V), diversity (D), joining (J), and constant region genes. In normal B cells these genes recombine via formation of double-stranded DNA breaks to generate functional *Ig* genes and protein with high antigen affinity. This process, known as V(D)J recombination, is the main mechanism for generating antigen-receptor diversity. The breaks and recombination events that occur during this process explain the tendency for chromosomal translocations in B-cell lymphomas to involve the Ig loci. However, the precise molecular mechanisms underlying these translocations remain unknown and different mechanisms have been proposed in various lymphoma types.

In this issue of *Blood*, Murga Penas and colleagues show that the t(14;18)(q32;q21) is the result, at least in part, of illegitimate V(D)J recombination.[4] By analyzing the sequence of the breakpoints at the *IgH* locus on chromosome 14, they identified findings typical of V(D)J-mediated recombination, that is, presence of a recombination signal sequence and evidence of coding end processing, including nucleotide deletions and insertion of non–template-dependent (N) nucleotides. The authors also found insertions of templated (T)–nucleotides at the junction sites. T-nucleotides are short copies of at least 5 nucleotides copied from the regions surrounding the breakpoints and often contain point mutations and/or insertions or deletions. Because T-nucleotides have not been described in normal V(D)J recombination products, their presence suggests that they are generated by error-prone template-dependent DNA synthesis rather than illegitimate V(D)J recombination. In contrast, analysis of the chromosome 18 breakpoints showed findings inconsistent with V(D)J recombination mechanisms. The authors identified an 87 base pair (bp) region in which the breakpoints of all cases clustered in the 5′ noncoding region of *MALT1*. In at least one case, analysis of the DH-MALT1 junction showed a duplication of 8 MALT1 nucleotides, suggesting a staggered double-stranded DNA break. Similar findings have been found in other chromosomal translocations, such as t(14;18)/*IgH-BCL2* in follicular lymphoma and t(11;14)/*CCND1-IgH* in mantle cell lymphoma, suggesting that these chromosomal translocations are generated by similar mechanisms.[5,6] In these translocations, illegitimate V(D)J mechanisms mediate recombination at the *IgH* locus, T-nucleotides are reported at the breakpoints, and breakpoint clusters occur in the *BCL2* and *CCND1* loci.

Another interesting implication of this study is the timing of translocations: when in B-cell differentiation do they occur? It seems intuitive that a particular chromosomal translocation may arise at a specific stage of B-cell differentiation, and therefore contributes to the biologic and clinical features of the neoplasm. In the case of MALT lymphomas, it would be expected that chromosomal translocations arise after the B cell has encountered antigen within the primary site of disease (eg, stomach). In the cases analyzed, the locations of the few somatic mutations identified suggest that they most likely resulted from error-prone repair of end-joining, rather than from the process of somatic mutation. These cases suggest, therefore, that MALT lymphomas arise from a B cell that encountered antigen outside the context of the germinal center microenvironment.

The diagnosis of MALT lymphoma can be challenging for pathologists. The problems in the diagnosis are related in part to the small size of biopsy specimens and the lack of specific immunophenotypic markers useful for diagnosis. For this reason, the identification of a novel, 87-bp cluster region in the t(14,18)(q32;q21)/*IgH-MALT1* will facilitate the development of polymerase chain reaction assays useful for the diagnosis in both fresh and paraffin-embedded biopsy specimens and will be helpful for the evaluation of minimal residual disease.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● PHAGOCYTES & GRANULOCYTES

Comment on Loges et al, page 2264

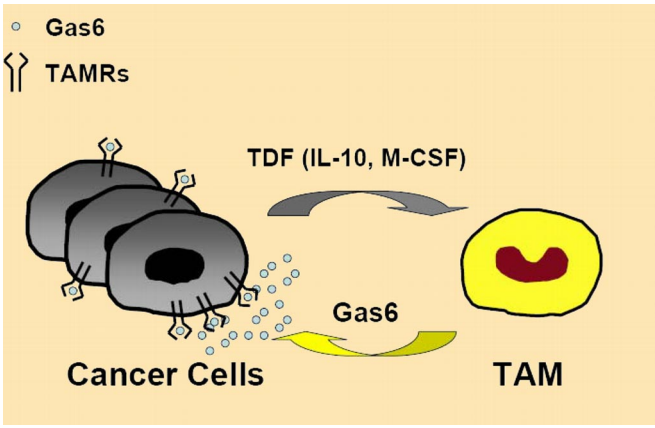
Macrophages give Gas(6) to cancer

Antonio Sica ISTITUTO CLINICO HUMANITAS

In this issue of *Blood*, Loges and colleagues have identified a novel tumor-promoting mechanism, whereby tumors educate tumor-associated macrophages to produce high levels of the mitogen Gas6, leading to tumor growth and metastasis.[1] Surprisingly, this newly identified protumoral activity of tumor-associated macrophages is restricted to the selective induction of cancer cell proliferation, without interfering with cancer cell survival, tumor-associated inflammation, and angiogenesis.

Gas6 is a member of the vitamin K–dependent ligand family, homologous to the blood coagulation protein S.[2] These molecules bind the family of receptor tyrosine kinases (TAMRs)—that includes Tyro-3, Axl, and Mer—and control various cellular functions, including macrophage clearance of apoptotic cells and natural killer cell differentiation.[3] Gas6 may also play a role as a key factor in cell survival[4] and platelet aggregation.[3,5]

Genetic events, including gene amplification, mutations, and altered protein expression, promote the oncogenic potential of TAMRs and have been found in various human cancers.[3] TAMRs display a certain degree of promiscuity and robustness, in that TAMR ligands share affinity for the entire family of receptor. Within this scenario, Gas6 has prominent affinity for Axl, which has transforming properties.[3] Contrasting evidence exists as to the prognostic significance of Gas6/Axl in cancer patients. Whereas increased Gas6/Axl interaction predicts poor prognosis in patients with glioblastoma and ovarian carcinoma,[6]



Tumor-derived factors (TDF), likely including IL-10 and M-CSF, educate TAM for Gas6 production to fuel cancer cell proliferation. TAMRs indicates TAM family of receptor tyrosine kinases; TAM, tumor-associated macrophages; M-CSF, macrophage colony-stimulating factor; and IL-10, interleukin-10.

an improved prognosis was observed in patients with renal cell carcinoma (RCC),[6] demonstrating the complexity of the system.

Loges et al provide new evidence on the regulation and significance of Gas6/Axl activity in cancer. Within the tumor microenvironment, tumor-associated macrophages acquire the capacity to express high levels of Gas6, suggesting that unidentified tumor-derived factor(s) contributes to their protumoral education.[7] Among the possible candidates, IL-10 and M-CSF were able to induce Gas6 upregulation in vitro. Interestingly, these factors promote M2 polarization of macrophages[7] and help shape the protumoral M2 phenotype (see figure) of tumor-associated macrophages.[7,8] It remains to be determined whether Gas6 represents a prototypical M2 marker and whether expression of high Gas6 levels is a feature of other tumor-associated myeloid cells, including myeloid-derived suppressor cells.[9]

Using different ectopic and orthotopic syngeneic tumor models, Loges et al demonstrate that inhibition of Gas6 does not influence accumulation of $CD45^+$ leukocytes, tumor-associated fibroblasts, angiogenesis, and coagulation, but it is limited to the selective inhibition of cancer cell proliferation. Further, the observed reduction in metastasis formation was secondary to reduced primary tumor growth. This scenario is rather surprising, as TAMRs are largely expressed by stromal components, including endothelial cells and leukocytes.[3]

The complex regulation of the Gas6 actions is highlighted by reports on its functional interplay with central regulators of inflammation and cell metabolism. Whereas activation of Gas6/Mer-dependent PI3K/akt pathway was reported to influence NF-κB activation,[3,10] alteration of the Von Hippel–Lindau tumor suppressor gene in clear cell renal cell carcinoma patients (ccRCC), a condition promoting a pseudohypoxic response and the consequent activation of the hypoxia-inducible factor-1 (HIF-1),[11] results in enhanced expression of Axl.[6] As tumor-associated macrophages accumulate in the hypoxic regions of a tumor[12] and activation of PI3K/Akt leads to HIF-1 activity,[13] it is tempting to speculate a possible link between the hypoxia/HIF-1 and Gas6/Axl pathways.

The contrasting information on the prognostic significance of Gas6/Axl in cancer, along with the multiple mechanisms involved in its regulation, suggest that the complexity of its biology is also contest- and tissue-specific. Although development of TAMRs antagonists may have therapeutic limitations, due to the possible induction of autoimmune disorders (eg, Lupus-like syndrome),[3] the cancer-restricted action of Gas6, along with its inertness on stroma cells, suggests that its therapeutic inhibition may well integrate strategies targeting cancer-related inflammation.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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● ● ● PLATELETS & THROMBOPOIESIS

Comment on Kasirer-Friede et al, page 2274

If Virchow were to meet Newton

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In this issue of *Blood*, Kasirer-Friede and colleagues show that ADAP is a component of a signaling system triggered when blood flow pulls αIIbβ3 bound to fibrinogen. It serves to convert tension into a biochemical response that stabilizes platelet attachment by directing lamellipodia formation.

Platelets are mechanical devices. Frictional forces generated by flowing blood induce an adhesive couple by altering the conformation of the extracellular domain of platelet glycoprotein (Gp) Ibα and by exposing the A1 domain of its ligand von Willebrand factor